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Technical Note

Performance of two swine manure treatment systems on chemical composition and on the reduction of pathogens

A. Viancelli [a], A. Kunz [b,*], R.L.R. Steinmetz [b], J.D. Kich [b], C.K. Souza [c], C.W. Canal [c], A. Coldebella [b], P.A. Esteves [b], C.R.M. Barardi [a]

[a] Departamento de Microbiologia e Parasitologia, Laboratório de Virologia Aplicada, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil
[b] Embrapa Suínos e Aves, 89700-000 Concórdia, SC, Brazil
[c] Laboratório de Virologia, Faculdade de veterinária, Universidade Federal do Rio Grande do Sul, 91540-000 Porto Alegre, RS, Brazil

HIGHLIGHTS

- Lagoon and compact treatment system reduce chemical and microbial from swine manure.
- Chemical and microbial reduction was better after lagoons treatment due to high HRT.
- Adenovirus showed to be a good environmental contamination marker.

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ABSTRACT

Swine effluents must be correctly handled to avoid negative environmental impacts. In this study, the profiles of two swine manure treatment systems were evaluated: a solid–liquid separation step, followed by an anaerobic reactor, and an aerobic step (System 1); and a biodigester followed by serial lagoons (System 2). Both systems were described by the assessment of chemical, bacterial and viral parameters. The results showed that in System 1, there was reduction of chemicals (COD, phosphorus, total Kjeldhal nitrogen – TKN – and NH_3), total coliforms and *Escherichia coli*; however, the same reduction was not observed for *Salmonella* sp. Viral particles were significantly reduced but not totally eliminated from the effluent. In System 2, there was a reduction of chemicals, bacteria and viruses with no detection of *Salmonella* sp., circovirus, parvovirus, and torque teno virus in the effluent. The chemical results indicate that the treated effluent can be reused for cleaning swine facilities. However, the microbiological results show a need of additional treatment to achieve a complete inactivation for cases when direct contact with animals is required.

1. Introduction

Swine production is a rapidly growing industry. This is especially true in Brazil, which is the fourth largest swine producer (3.36 Mt yr^{-1}), the fourth largest exporter (0.52 Mt yr^{-1}), and the sixth largest consumer (15 kg yr^{-1} $person^{-1}$) in the world (ABIPECS, 2011). Furthermore, there has been an increase of swine manure generation and swine-related water consumption. It is estimated that 6 m^3 of water is necessary to produce 1 kg of pork (Palhares, 2011).

Swine effluent contains pig urine, feces, water spillage, remains of undigested feed items, antimicrobial drug residues and pathogenic microorganisms. Considering these characteristics, it is recommended that this material be correctly managed before its application to land to avoid potential environmental contamination (Hundesa et al., 2009). Recent studies have proposed treatment strategies for swine manure that include physical, chemical and biological processes designed for the effective removal of organic compounds and the inactivation of bacteria (Vanotti et al., 2005; Costantini et al., 2007). In Brazil, the predominant manure management strategy currently adopted is pit storage followed by land application (Kunz et al., 2009). For treatment, the most commonly used option is the anaerobic treatment/covered lagoon system (Pérez-Sangrador et al., 2012).

Commonly, swine manure is characterized by a high content of suspended solids, organic matter, and high phosphorus and nitrogen contents (Steinmetz et al., 2009). Additionally, high levels of microbial populations are observed including total coliforms, *Escherichia coli*, and *Salmonella* sp. (Hutchison et al., 2005). Viruses as adenovirus, torque teno virus, parvovirus and circovirus have also been observed. These microorganisms are important when

* Corresponding author. Tel.: +55 49 3441 0400; fax: +55 49 3442 8559.
E-mail address: airton.kunz@embrapa.br (A. Kunz).

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considered the ramifications for both human and animal biosecurity (Martens and Böhm 2009).

Salmonella is a rod-shaped, Gram-negative bacteria, belonging to the genus *Salmonella* (*S.*), family Enterobactereaceae. *Salmonella* colonizes the intestinal tract of animals and humans. Over 2500 serovars have been classified according to antigen composition. Animals can be infected with a wide variety of serovars that may or may not clinically manifest in the host (Griffith et al., 2012). Coliforms are a group of bacteria functionally-related that belong to different genera (*Echerichia, Citrobacter, Enterobacter* and *Klebsiella*), where 80% of coliform bacteria are represented by *E. coli* and are used as biological indicator of the sanitary quality for water and food (Tortora et al., 2005).

Porcine adenovirus (PAdV), porcine circovirus (PCV2), porcine parvovirus (PPV1) and torque teno virus (TTV) are non-enveloped DNA viruses that have been reported to be widespread within swine populations (Hundesa et al., 2009; Shangjin et al., 2009). PCV2 is associated with Post-weaning Multisystemic Wasting Syndrome (PMWS), and PPV causes reproductive failure in swine (Shangjin et al., 2009).

In contrast with the swine production, the environmental legislation regarding the security parameters is recent. In Brazil, the Resolution CONAMA 430 (CONAMA, 2011) is used to guide the discharge on effluent in water bodies. However, nothing has been established about the security parameters for the water reuse on animal production. Concerning the described above and the reusing of water from treated manure, the present work aimed to evaluate the water quality from two distinct swine manure treatment systems considering the capacity on abatement of chemical and microbiological parameters.

2. Materials and methods

2.1. Treatment systems

The facilities were located at Embrapa Swine and Poultry, Concórdia, SC, Brazil. System 1 received piggery wastewater from Embrapa's experimental facilities (15 $m^3\ d^{-1}$). The treatment system consisted of a solid–liquid separation step using a screen, an equalization tank, a settling tank, an anaerobic reactor, an aerobic reactor and a second settling tank (Kunz et al., 2009). System 2 consisted of a anaerobic digester followed by serial lagoons (anaerobic, facultative, and maturation). (Techio et al., 2011). The schematic representation of both systems is shown in Fig. 1.

2.2. Manure sampling sites

A total of 86 piggery samples from the two manure management treatment systems were collected from March 2009 to December 2010. The samples were collected once a month (except in Oct/09, Dec/09, Jan/10 and Feb/10 (both systems) and May/10, June/10, September/10 and October/10 (System 2) due to systems operational problems. The sampling sites in the System 1 were located as follows: site 1 after the equalization tank (representing the raw manure), site 2 after the solid–liquid separation, and site 3 represented the treated wastewater (after the biological steps). Sampling sites in the System 2 were located before (site 1) and after (site 2) anaerobic digester, site 3 after the maturation lagoon to represent the treated wastewater. All the sampling events were performed at the same day in the morning.

2.3. Sample storage and chemical analysis

1 L of each sample was collected in a polyethylene flask and stored at 4 °C before analysis COD, total phosphorus (TP), nitrogen (TKN and NH_3) were determined according to APHA (2005).

2.4. Bacterial analysis

Total coliforms and *E. coli* analysis were performed using the Petrifilm *E. coli*/Coliform Count Plate kit (USA), following the manufacturer's instructions. *Salmonella* quantitative detection was obtained using the Most Probable Number (MPN) assay, performed according to Bacteriological Analytical Manual (BAM, 2003).

2.5. Viral analysis

20 mL samples were collected from each site. Samples were concentrated, and submitted to DNA extraction as described by Viancelli et al. (2011). For PPV, TTV, PAdV and PCV2 detection, DNA was submitted to qualitative PCR (qPCR) following the protocols described by Soares et al. (1999), Segalés et al. (2009), Hundesa et al. (2009) and Viancelli et al. (2011), respectively. In the case of PAdV and PCV2 reactions, qPCR positive samples were

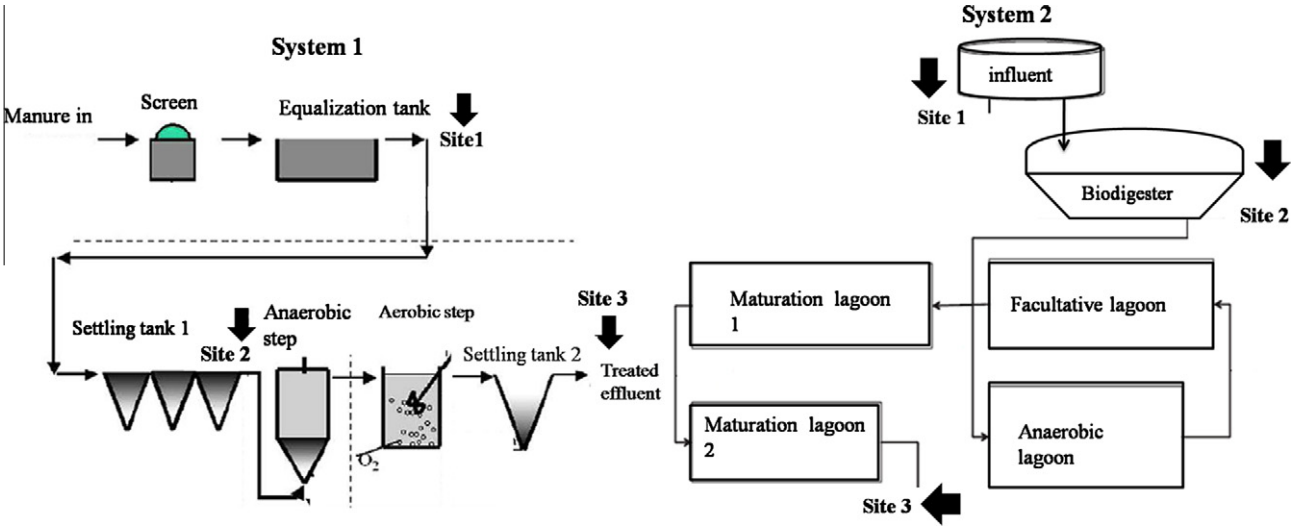

Fig. 1. Schematic representation of the two swine manure treatment systems analyzed in the present study. The sampling sites are indicated on both systems are indicated by black arrows. The sites represent the influent, the intermediate and the final effluent.

submitted to DNAse treatment to assess the viral integrity of the samples (Viancelli et al., 2011).

2.6. Statistical analysis

The statistical analyses were performed using a GLM variance analysis conducted with Manual SAS software (2003). For *Salmonella* sp. and viral statistical analysis, the Fisher exact test was performed (SAS, 2003).

3. Results

3.1. System 1

According to the results displayed in Table 1, the overall reduction of COD, TKN, TP and NH_3 observed for treatment System 1 was statistically significant ($p < 0.05$). The chemical profile showed a 39% reduction of COD after solid–liquid separation, 56% after the biological step and an overall COD reduction of 95% throughout the system. The efficiency of TKN and NH_3–N removal was poor in the solid–liquid separation step (20% and 5%, respectively) but was statistically significant in the biological treatment step (58% and 70%, respectively). The overall efficiency of reduction for N species was 78% for TKN and 75% for NH_3. In contrast, TP was removed more efficiently in the solid–liquid separation step (43%) than in the biological steps (31%).

The bacterial profile showed a significant log reduction of total coliforms, decreasing from 5.04 ± 0.06 in the raw manure to 2.97 ± 0.15 log CFU mL^{-1} in the final effluent. The *E. coli* analyses showed a significant reduction, which went from 4.77 ± 0.15 to 2.31 ± 0.31 log CFU mL^{-1} (Fig. 2a). The analysis of *Salmonella* sp. showed a log average of 0.34 ± 0.18, 0.74 ± 0.27 and 0.28 ± 0.13 log MPN mL^{-1} in the raw manure, the physically treated manure, and the final effluent, respectively (Fig. 2b).

Table 1
Average of each chemical parameter analyzed from March 2009 to December 2010 in System 1. Significance to $p \leqslant 0.05$.

Parameter	Raw manure (site 1)	After solid–liquid step (site 2)		After biological step (site 3)		Global efficiency (%)
	Mean (SD[a]) ($g\ L^{-1}$)	Mean (SD) ($g\ L^{-1}$)	Step efficiency[b]/global efficiency contribution (%)	Mean (SD) ($g\ L^{-1}$)	Step efficiency/global efficiency contribution (%)	
COD	22.929 (±1.889) a	13.906 (±1.785) b	39/39	1.145 (±0.103) c	92/56	95
TKN	2.023 (±0.154) a	1.622 (±0.133) b	20/20	0.438 (±0.113) c	73/58	78
NH_3-N	1.207 (±0.111) a	1.144 (±0.097) a	5/5	0.300 (±0.071) b	74/70	75
TP	0.358 (±0.033) a	0.204 (±0.029) b	43/43	0.094 (±0.007) c	54/31	74

[a] SD: standard deviation.
[b] Efficiency = $(X_0 - X)/X_0 \cdot 100$; where X_0 = initial concentration, X = final concentration.

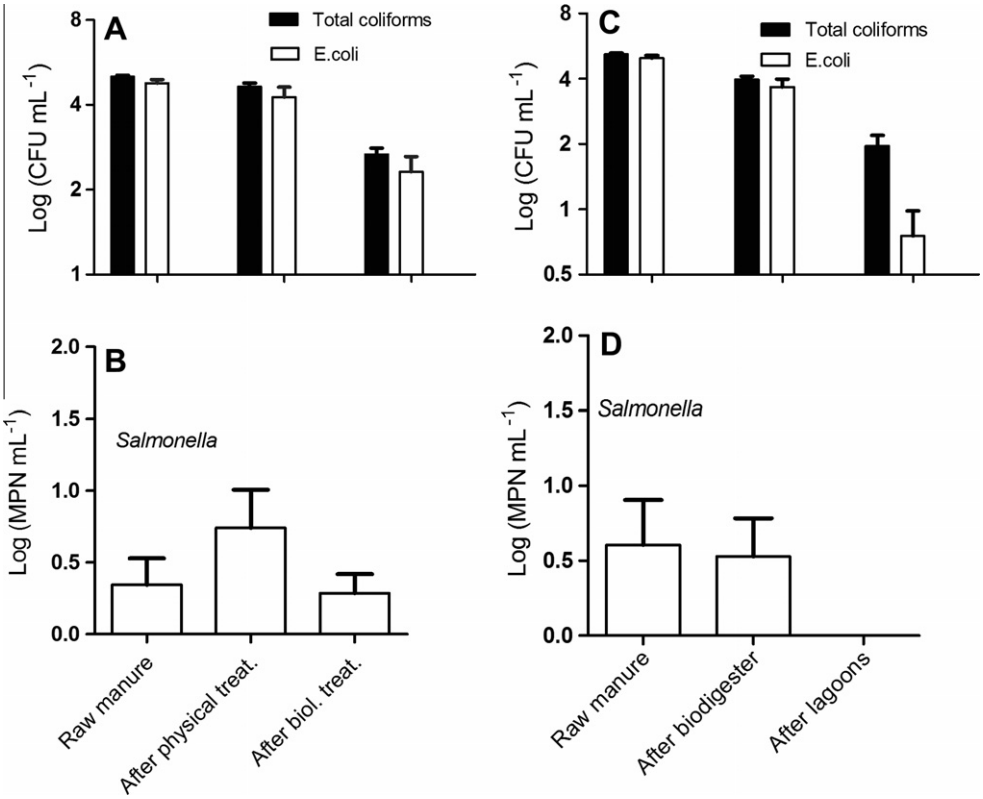

Fig. 2. Average log reduction of CFU mL^{-1} of total coliforms (black bars) and *E. coli* (white bars); average log reduction on MPN mL^{-1} and percentage of positive samples for *Salmonella* sp. at each site from System 1 (a–c) and at each site from System 2 (d–f) from March 2009 to December 2010.

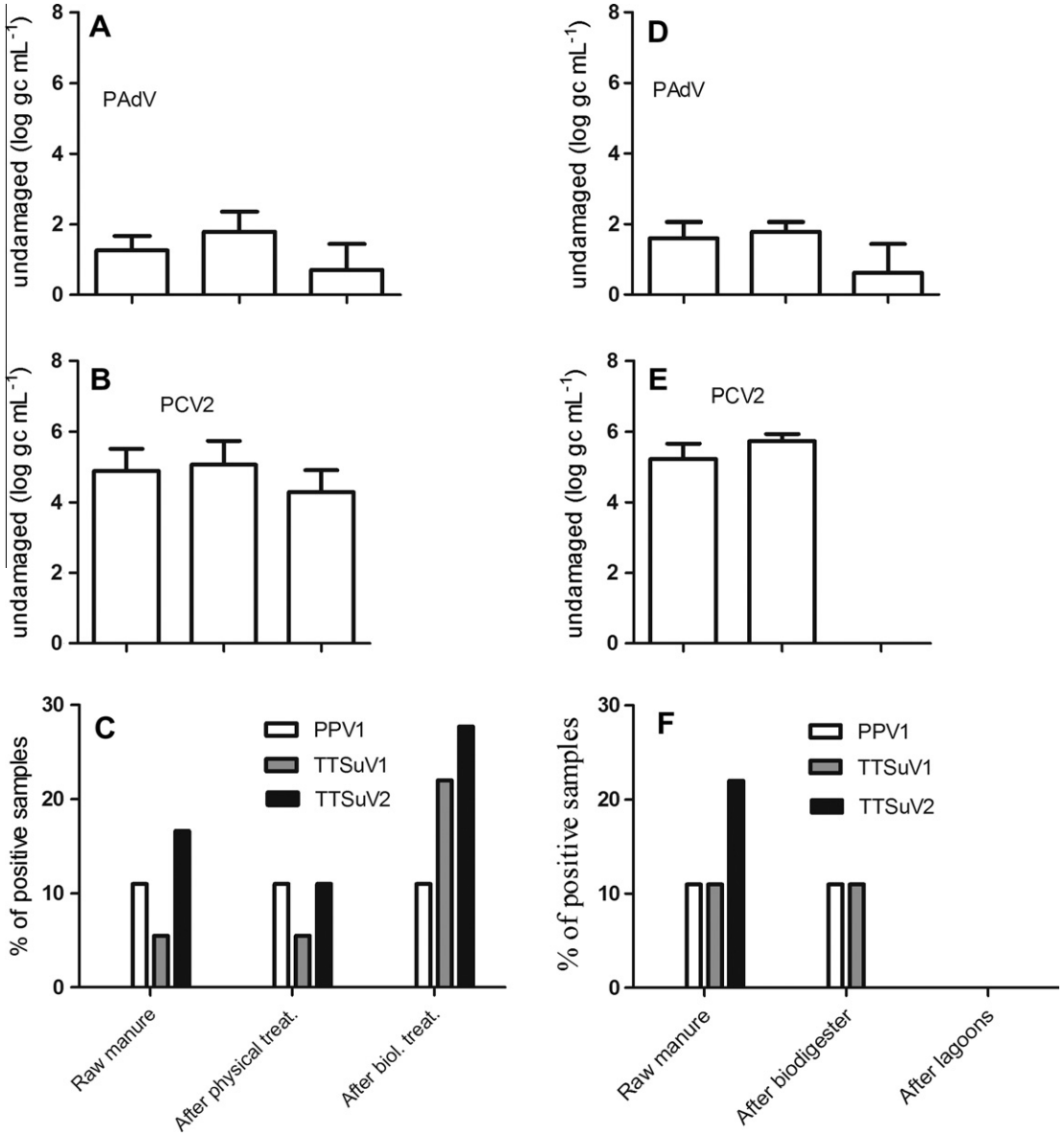

Fig. 3. Percentage of positive PPV1, TTV1 and TTV2 samples; average number of gc mL^{-1} of undamaged PAdV and PCV2 virus particles in System 1 (a–c) and in System 2 (d–f).

Fig. 3a illustrates the results for PAdV, where the amount of gc mL^{-1} of undamaged PAdV presented 1 log (90%) reduction after manure treatment (final effluent). In contrast, no reduction of undamaged PCV2 was observed along the treatment process (Fig. 3b). Fig. 3c illustrates the results for TTV1, TTV2 and PPV1. TTV1 genomes were positive in 5%, 5% and 22% of the samples from raw manure, after physical treatment and final effluent, respectively. In contrast, TTV2 genomes were positive in 16%, 11% and 27% of the samples from raw manure, after physical treatment and final effluent, respectively. The percentage of PPV1 positive samples was the same in the raw manure, after physical treatment and in the final effluent (11%).

3.2. System 2

Table 2 displays the chemical parameters for System 2 in the studied samples. According to these data, it was observed that the sludge was more concentrated than raw manure. The data in Table 2 show a similar efficiency of COD reduction after the biodigester (47%) and lagoons (52%). COD was the only parameter that presented a significant reduction during the biodigester step. The reductions observed for TKN, TP and NH_3 in the manure before and after the biodigester were not statistically significant.

The bacterial profile showed a significant log reduction of total coliforms. Levels decreased from 5.18 ± 0.07 in the raw manure to 1.96 ± 0.23 log CFU mL^{-1} in the final effluent. The *E. coli* analyses showed a significant log reduction, which was from 4.97 ± 0.14 to 0.75 ± 0.23 log CFU mL^{-1} (Fig. 2c). The analysis of *Salmonella* sp. showed 0.60 ± 0.30, 0.53 ± 0.25 log MPN mL^{-1} in the sludge before and after the anaerobic biodigestion respectively (Fig. 2d). No positive samples were found in the final effluent (Fig. 2d).

The results for TTV1, TTV2 and PPV1 are shown in Fig. 3f. PPV1 and TTV1 were positive in the raw manure (11%) and in the biodigester effluent (11%) but were absent in the final effluent. TTV2 was positive only in the raw manure (22%). The undamaged PAdV reduction was 1 log, and undamaged PCV2 was absent in the final effluent (Fig. 3d and e, respectively).

4. Discussion

The most common destination of manure worldwide is the land application; however pathogens present in manure can affect soil

Table 2
Average of each chemical parameter analyzed from March 2009 to May 2010 in System 2. Significance to $p \leqslant 0.05$.

Parameter	Sludge (site 1)	After biodigester (site 2)		After lagoons (site 3)		Global efficiency (%)
	Mean (SD[a]) ($g\ L^{-1}$)	Mean (SD) ($g\ L^{-1}$)	Step efficiency[b]/global efficiency contribution (%)	Mean (SD) ($g\ L^{-1}$)	Step efficiency/global efficiency contribution (%)	
COD	64.310 (±10.380) a	34.285 (±4.036) b	47/47	0.857 (±0.215) c	97/52	99
TKN	3.334 (±0.425) a	2.681 (±0.267) a	20/20	0.170 (±0.030) b	94/75	95
NH_3-N	1.195 (±0.129) a	0.986 (±0.100) a	17/17	0.160 (±0.037) b	84/69	86
TP	1.071 (±0.175) a	1.191 (±0.196) a	<1/<1	0.015 (±0.003) b	98/98	98

[a] SD: standard deviation.
[b] Efficiency = $(X_0 - X)/X_0 \cdot 100$; where X_0 = initial concentration, X = final concentration.

and water quality, as well as the health of animals and humans working in livestock production (Kunz et al., 2009).

The chemical profile of the systems in the present study was similar to that observed by Steinmetz et al. (2009), which studied the System 1 and the influent from System 2. In that study, the insoluble species (e.g., organic nitrogen and TP, metals and other inorganic species) were removed more efficiently by a solid–liquid separation step. The low *N* removal capacity in the solid–liquid separation suggests a degraded effluent with an ammonification process contribution in the pits (Ndegwa et al., 2002). Organic carbon was easily removed by anaerobic digestion. The NH_3, which is highly soluble, was not removed by solid–liquid separation but greatly removed during aerobic process.

A significant nitrogen reduction was observed after the lagoon step (System 2) and this profile agrees with data reported by Vivan et al. (2010). The reduction of nitrogen species possibly occurred because of biological processes (e.g., nitrification/denitrification), algal fixation or ammonia volatilization. The results for TP obtained in the present study (P reduction from >1 g L^- to <0.02 g L^-) can be explained by the generation of insoluble phosphorus compounds that have aggregated in the sediment and precipitated. According previously reported by Fernandes et al. (2012), the phosphorus can be removed by precipitation, mainly after biological treatment. The System 2 has a hydraulic retention time (HRT) around 170 d (Vivan et al., 2010), that considered a large time to P removal due to combination with calcium ions present in effluent and subsequent precipitation.

Total coliform and *E.coli* reduction was expected once the organic material reduction during the process decreased the fecal indicators due to an increase in microbiological competition for substrate. However, *Salmonella* did not show this association, and the higher number of positive samples found in the final effluent (System 1) when compared with the raw manure might be explained by system dynamics fluctuations. Other potential factors are that *Salmonella* excretion might be intermittent; there could also be a competitive process between *Salmonella* sp. and other bacteria. During the treatment process, competing bacteria could potentially be eliminated, thus allowing *Salmonella* sp. to predominate.

Recent studies have shown that *Salmonella* sp. is frequently recovered from swine manure (Hutchison et al., 2005). Biological aerobic and anaerobic processes are capable of inactivating microorganisms, and the efficiency of pathogen inactivation is related to different factors such as antibiosis, redox-potential, antagonism, nutrient deficiencies and exothermic metabolism. However, the most effective factor in this context is temperature. Generally, only thermophilic processes are suitable for the inactivation of pathogens because bacteria are inactivated within a reasonable timeframe (Martens and Böhm, 2009). The anaerobic digestion does not produce enough heat to inactivate bacteria. The UASB (System 1) and the biodigester (System 2) do not have heating systems and in tropical (15–38 °C) and subtropical (10–32 °C) areas as Brazil are limited to environmental temperatures (Kunz et al., 2005).

All viruses analyzed in the present study were resistant to the treatment processes employed. TTV is highly prevalent in piglets displaying PMWS (Shangjin et al., 2009). However, there are no other studies reporting TTV as it relates to swine manure treatment. In contrast, other studies reported human torque teno virus DNA in influent and effluent samples collected from a wastewater treatment plant in Japan (Haramoto et al., 2008). Therefore, TTV may be an efficient indicator system for viral pathogen risk for drinking water utilities, watershed managers, and protection agencies (Griffin et al., 2008). However, other studies reported that TTV is not a good fecal contamination marker (Hamza et al., 2011).

PCV2 and PAdV were more prevalent than other viruses and can possibly be considered as indicators of manure contamination. Therefore, it could be suggested that PAdV be used as a viral marker of swine manure contamination in environmental samples. This choice is based on the resistance of this virus, as the results showed the PAdV was the only virus present in the final effluent from System 2, once this virus is eliminated probably all other will be.

The significant reduction of all parameters analyzed after the lagoon treatment suggest that this can be a good alternative for swine manure treatment, although the major disadvantage of this approach is a very high HRT. Nevertheless, bacteria and viruses were not completely eliminated. One option to ameliorate this problem would be the addition of an inactivation process after the last lagoon, such as pH elevation (>10) by the application of lime (Vanotti et al., 2005). Physical and chemical agents can also be applied and the success of bacteria and viruses inactivation is strongly related to the removal of organic material, which at high concentrations makes the action of agents as UV light and chlorides difficult (Olson et al., 2004). Sahlström et al. (2008) studied the pasteurization of swine manure for 60 min at 70 °C and verified that this was not enough time or a high enough temperature to inactivate viruses such as PPV.

All the results obtained can be used as a guide for future discussion about water reuse politics. Likewise, for a water reuse in the barns and direct contact with animals, an additional inactivation step (i.e. pH increase, UV, chlorination) should be added to ensure the biosecurity level.

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